

Attorney Docket No.: DEX-0315
Inventors: Salceda et al.
Serial No.: 10/076,747
Filing Date: February 13, 2002
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Please amend the specification as follows:

Please replace the paragraph at page 35, line 31, through page 36, line 12, with the following:

Commercially available fluorescent nucleotide analogues readily incorporated into the nucleic acids of the present invention include Cy3-dCTP, Cy3-dUTP, Cy5-dCTP, Cy3-dUTP (Amersham Biosciences, Piscataway, New Jersey, USA), fluorescein-12-dUTP, tetramethylrhodamine-6-dUTP, ~~Texas Red~~ TEXAS RED® (red fluorescent dye) -5-dUTP, ~~Cascade Blue~~ CASCADE BLUE (blue fluorescent dye)®-7-dUTP, BODIPY® (fluorescent dyes with narrow emission bandwidths with minimal spectral overlap for multicolor applications) FL-14-dUTP, BODIPY® TMR-14-dUTP, BODIPY® TR-14-dUTP, Rhodamine Green™-5-dUTP, ~~Oregon Green~~ OREGON GREEN (green fluorescent dye)® 488-5-dUTP, ~~Texas Red~~ TEXAS RED®-12-dUTP, BODIPY® 630/650-14-dUTP, BODIPY® 650/665-14-dUTP, ~~Alexa Fluor~~ ALEXA FLUOR ® (fluorescent dye that spans the visible spectrum) 488-5-dUTP, ~~Alexa Fluor~~ ALEXA FLUOR® 532-5-dUTP, ~~Alexa Fluor~~ ALEXA FLUOR® 568-5-dUTP, ~~Alexa Fluor~~ ALEXA FLUOR® 594-5-dUTP, ~~Alexa Fluor~~ ALEXA FLUOR® 546-14-dUTP, fluorescein-12-UTP, tetramethylrhodamine-6-UTP, ~~Texas Red~~ TEXAS RED®-5-UTP, ~~Cascade Blue~~ CASCADE BLUE®-7-UTP, BODIPY® FL-14-UTP, BODIPY® TMR-14-UTP, BODIPY® TR-14-UTP, Rhodamine Green™-5-UTP, ~~Alexa Fluor~~ ALEXA FLUOR® 488-5-UTP, ~~Alexa Fluor~~ ALEXA FLUOR® 546-14-UTP (Molecular Probes, Inc. Eugene, OR, USA). One may also custom synthesize nucleotides having other fluorophores. See Henegariu et al., Nature Biotechnol. 18: 345-348 (2000).

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Please replace the paragraph at page 53, lines 15-31, with the following:

Polypeptides of the invention may be post-translationally modified. Post-translational modifications include phosphorylation of amino acid residues serine, threonine and/or tyrosine, N-linked and/or O-linked glycosylation, methylation, acetylation, prenylation, methylation, acetylation, arginylation, ubiquination and racemization. One may determine whether a polypeptide of the invention is likely to be post-translationally modified by analyzing the sequence of the polypeptide to determine if there are peptide motifs indicative of sites for post-translational modification. There are a number of computer programs that permit prediction of post-translational modifications. See, e.g., www.expasy.org expasy.org of the world wide web (accessed August 31, 2001), which includes PSORT, for prediction of protein sorting signals and localization sites, SignalP, for prediction of signal peptide cleavage sites, MITOPROT and Predotar, for prediction of mitochondrial targeting sequences, NetOGlyc, for prediction of type O-glycosylation sites in mammalian proteins, big-PI Predictor and DGPI, for prediction of prenylation-anchor and cleavage sites, and NetPhos, for prediction of Ser, Thr and Tyr phosphorylation sites in eukaryotic proteins. Other computer programs, such as those included in GCG, also may be used to determine post-translational modification peptide motifs.

Please replace the paragraph at page 53, line 32, through page 54, line 10, with the following:

General examples of types of post-translational

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modifications may be found in web sites such as the Delta Mass database <http://www.abrf.org/ABRF/ResearchCommittees/deltamass/deltamass.html> [abrf.org/ABRF/ResearchCommittees/deltamass/deltamass.html](http://www.abrf.org/ABRF/ResearchCommittees/deltamass/deltamass.html) of the world wide web (accessed October 19, 2001); "GlycoSuiteDB: a new curated relational database of glycoprotein glycan structures and their biological sources" Cooper et al. Nucleic Acids Res. 29: 332-335 (2001) and <http://www.glycosuite.com/> [glycosuite.com](http://www.glycosuite.com/) of the world wide web (accessed October 19, 2001); "O-GLYCBASE version 4.0: a revised database of O-glycosylated proteins" Gupta et al. Nucleic Acids Research, 27: 370-372 (1999) and <http://www.cbs.dtu.dk/databases/OGLEYCBASE/> [cbs.dtu.dk/databases/OGLEYCBASE/](http://www.cbs.dtu.dk/databases/OGLEYCBASE/) of the world wide web (accessed October 19, 2001); "PhosphoBase, a database of phosphorylation sites: release 2.0.", Kreegipuu et al. Nucleic Acids Res 27(1):237-239 (1999) and <http://www.cbs.dtu.dk/databases/PhosphoBase/> [cbs.dtu.dk/ databases/PhosphoBase/](http://www.cbs.dtu.dk/databases/PhosphoBase/) of the world wide web (accessed October 19, 2001); or <http://pir.georgetown.edu/pirwww/search/textresid.html> [pir.georgetown.edu/ pirwww/search/textresid.html](http://pir.georgetown.edu/pirwww/search/textresid.html) of the world wide web (accessed October 19, 2001).

Please replace the paragraph at page 56, lines 3-24 with the following:

In another embodiment, the invention provides polypeptides that have been post-translationally modified. In one embodiment, polypeptides may be modified enzymatically or chemically, by addition or removal of a post-translational modification. For example, a polypeptide may be glycosylated or deglycosylated

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enzymatically. Similarly, polypeptides may be phosphorylated using a purified kinase, such as a MAP kinase (e.g., p38, ERK, or JNK) or a tyrosine kinase (e.g., Src or erbB2). A polypeptide may also be modified through synthetic chemistry. Alternatively, one may isolate the polypeptide of interest from a cell or tissue that expresses the polypeptide with the desired post-translational modification. In another embodiment, a nucleic acid molecule encoding the polypeptide of interest is introduced into a host cell that is capable of post-translationally modifying the encoded polypeptide in the desired fashion. If the polypeptide does not contain a motif for a desired post-translational modification, one may alter the post-translational modification by mutating the nucleic acid sequence of a nucleic acid molecule encoding the polypeptide so that it contains a site for the desired post-translational modification. Amino acid sequences that may be post-translationally modified are known in the art. See, e.g., the programs described above on the website www.expasy.org expasy.org of the world wide web. The nucleic acid molecule is then be introduced into a host cell that is capable of post-translationally modifying the encoded polypeptide. Similarly, one may delete sites that are post-translationally modified by either mutating the nucleic acid sequence so that the encoded polypeptide does not contain the post-translational modification motif, or by introducing the native nucleic acid molecule into a host cell that is not capable of post-translationally modifying the encoded polypeptide.

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Please replace the paragraph at page 58, line 28, through page 59, line 8 with the following:

Plasmid vectors will typically be introduced into chemically competent or electrocompetent bacterial cells. *E. coli* cells can be rendered chemically competent by treatment, e.g., with CaCl_2 , or a solution of Mg^{2+} , Mn^{2+} , Ca^{2+} , Rb^+ or K^+ , dimethyl sulfoxide, dithiothreitol, and hexamine cobalt (III), Hanahan, *J. Mol. Biol.* 166(4):557-80 (1983), and vectors introduced by heat shock. A wide variety of chemically competent strains are also available commercially (e.g., Epicurian Coli® XL10-Gold® Ultracompetent Cells (Stratagene, La Jolla, CA, USA); DH5 α competent cells (Clontech Laboratories, Palo Alto, CA, USA); and TOP10 Chemically Competent *E. coli* Kit (Invitrogen, Carlsbad, CA, USA)).

Bacterial cells can be rendered electrocompetent, that is, competent to take up exogenous DNA by electroporation, by various pre-pulse treatments; vectors are introduced by electroporation followed by subsequent outgrowth in selected media. An extensive series of protocols is provided online in Electroprotocols (BioRad, Richmond, CA, USA)

~~(http://www.biorad.com/LifeScience/pdf/New_Gene_Pulser.pdf~~
~~biorad.com/LifeScience/pdf/New_Gene_Pulser.pdf of the world wide web).~~

Please replace the paragraph at page 59, line 31 through page 60, line 15 with the following:

Mammalian and insect cells can be directly infected by packaged viral vectors, or transfected by chemical or electrical means. For chemical transfection, DNA can be coprecipitated with CaPO_4 , or introduced using liposomal and nonliposomal lipid-based

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agents. Commercial kits are available for CaPO₄ transfection (CalPhos™ Mammalian Transfection Kit, Clontech Laboratories, Palo Alto, CA, USA), and lipid-mediated transfection can be practiced using commercial reagents, such as LIPOFECTAMINE™ 2000, LIPOFECTAMINE™ Reagent, CELLFECTIN® Reagent, and LIPOFECTIN® Reagent (Invitrogen, Carlsbad, CA, USA), DOTAP Liposomal Transfection Reagent, FuGENE 6, X-tremeGENE Q2, DOSPER, (Roche Molecular Biochemicals, Indianapolis, IN USA), Effectene™, PolyFect®, Superfect® (Qiagen, Inc., Valencia, CA, USA). Protocols for electroporating mammalian cells can be found online in Electroprotocols (Bio-Rad, Richmond, CA, USA) (http://www.bio-rad.com/LifeScience/pdf/New_Gene_Pulser.pdf [bio-rad.com/LifeScience/pdf/New_Gene_Pulser.pdf](http://www.bio-rad.com/LifeScience/pdf/New_Gene_Pulser.pdf) of the world wide web); Norton et al. (eds.), Gene Transfer Methods: Introducing DNA into Living Cells and Organisms, BioTechniques Books, Eaton Publishing Co. (2000); incorporated herein by reference in its entirety. Other transfection techniques include transfection by particle bombardment and microinjection. See, e.g., Cheng et al., *Proc. Natl. Acad. Sci. USA* 90(10): 4455-9 (1993); Yang et al., *Proc. Natl. Acad. Sci. USA* 87(24): 9568-72 (1990).

At page 115, line 20, please insert the following paragraph:
The deposit was made with the American Type Culture Collection at 1801 University Boulevard in Manassa, Virginia 20110-2209.